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# Occurrence and postharvest strategies to help mitigate aflatoxins and fumonisins in maize and their co-exposure to consumers in Mexico and Central America

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ABSTRACT

Maize is the main dietary cereal in Mexico and Central America, with annual per capita consumption between 25.5 and 116.34 kg. Unfortunately, maize is highly susceptible to fungal infestation in the field, either through systemic infections or wounds caused by farm equipment, birds or insects. Field infestations can be exacerbated by bad postharvest handling practices. Proliferation of fungi on maize grains can alter physical appearance, taste and chemical composition, including accumulation of toxic fungal metabolites known as mycotoxins. Such metabolites can also be found in other crops that are also essential in the diet of the population in this region, including beans, rice and chili peppers.

Maize grown in Mexico and Central America is mainly contaminated by mycotoxins belonging to the aflatoxins (AFs) and fumonisins (FBs) groups, produced by the fungi *Aspergillus* and *Fusarium*, respectively. These mycotoxins are of public health concern because they can induce negative health impacts including cancer in humans and animals. AFs and FBs levels of up to 2630 and 3861 μg/kg, respectively, have been reported in the region between 2017 and 2021. These levels are more than 380 times higher than established maximum levels.

Pre- and post-harvest strategies can help mitigate mycotoxin contamination of grain. Pre-harvest AFs and FBs management strategies include the use of tolerant germplasm, good agronomic management, and biological control. Post-harvest strategies include all practices from harvest until consumption.

This review examines AFs and FBs predisposing factors, prevalence, and co-occurrence in Mexico and Central America. We discuss common post-harvest practices, and recommended practices to reduce mycotoxin contamination, including optimum grain drying (to decrease moisture content below 14%); grain sorting (with the potential to reduce AFs and FBs levels by 40–95%); use of grain conditioning agents, grain quality-management, and hermetic storage technologies and optimization of storage conditions. The effects of grain processing, including baking, roasting, popping, and nixtamalization on reducing AFs and FBs (15–80% for AFs, 17–100% for FBs) are also reviewed. This review highlights the widespread mycotoxin contamination problem and the urgent need for new research paradigms to inform mycotoxin mitigation strategies in the region.

## 1. Introduction

Maize is the third most important food crop in the world after wheat and rice, but the first in annual production by volume. The average daily consumption of maize products by Mexican and Central American populations is 319 g and 282 g respectively. These intakes provide approximately 986 kcal and 25.4 g of protein for Mexicans and 888 kcal and 22.9 g of protein for Central Americans (FAOSTAT, 2021). People in Mexico and Central American countries mainly consume maize in the form of tortillas and related nixtamalized maize-based food products (Table 1). Nixtamalization is a traditional cooking method in which maize is cooked and steeped in an alkaline solution (Escalante-Aburto, Mariscal-Moreno, Santiago-Ramos, & Ponce-García, 2020).

During the growth of the maize plant and during postharvest activities, kernels may get colonized by an array of fungi, some of them producing low molecular-weight secondary metabolites known as

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mycotoxins. Maize is generally susceptible to infection by fungal genera like *Aspergillus, Fusarium* and *Penicillium* where they can produce different symptoms in the kernels and plants or remain symptomless (Fig. 1; Table 2). Members of these genera produce mycotoxin groups such as aflatoxins (AFs), fumonisins (FBs), deoxynivalenol (DON), and other trichothecenes and zearalenone (ZEN) (Munkvold, Arias, Taschl, & Gruber-Dorninger, 2019). The risk of infection and subsequent contamination with mycotoxins increases when the crops grow under abiotic stress such as high temperature or drought. Several other crops, including beans, wheat, rice, chili peppers and peanuts that are also part of the Mexican and Central American diet are affected by mycotoxins (Bandyopadhyay et al., 2016; Costa et al., 2019; Telles, Kupski, & Furlong, 2017; Voth-Gaeddert, Stoker, Torres, & Oerther, 2020). In fact, maize-based products are commonly consumed with beans and chilies and are the bases of the diet, especially in rural areas (Table 1).

Direct exposure by humans and animals to mycotoxins through consumption of contaminated food and feed can result in toxic health effects (Alshannaq & Yu, 2017; Martínez Padrón, Hernández Delgado, Reyes Méndez, & Vázquez Carrillo, 2013). If maize is a dietary staple, as is the case in the region, the contamination could translate to high-level chronic or acute exposure. In Mexico and Central America, the effects are more chronic, while in parts of Africa like Kenya, Tanzania, Nigeria or in Asia, e.g., in India, both chronic and acute exposure have been reported (Granados-Chinchilla et al., 2017; Kumar, Mahato, Kamle, Mohanta, & Kang, 2017; Mahuku et al., 2019; Morales-Moo et al., 2020; Voth-Gaeddert et al., 2020). Mycotoxins therefore pose a threat to food safety, food security and international trade (WHO, 2021).

In the past 10 years, AFs outbreaks have been witnessed in regions such as East Africa and India. On the contrary, no AF or any other mycotoxin outbreaks have been reported in Mexico or countries in Central America (Kumar et al., 2017; Mahuku et al., 2019). However, scientific reports in countries of this region during the same period indicate that mycotoxin contamination in grain is an emerging concern. For instance, according to the BIOMIN mycotoxin survey data on maize, other cereals and finished products intended for feed from Latin America between 2017 and 2021 there is rampant exposure to 4 main mycotoxins AF, FB, DON and ZEN. During this period up to 27% of samples analyzed tested positive to AF with lowest positive annual average value of 4 μg/kg and a maximum of 3861 μg/kg. Comparatively FB were detected in up to 90% of samples analyzed with the lowest positive average of 1390 μg/g and a maximum detected level of 21883 μg/kg. DON was detected in up to 82% of total samples analyzed with the lowest average of 340 μg/kg in positive samples and the maximum detectable level of 26 320 μg/kg. Finally, ZEN was detected in up to 60% of samples analyzed during the period where it returned the lowest positive average of 53 μg/kg and maximum detectable level of 4948 μg/kg. It is important to note that the total average exposure factor which is the probability that one would be exposed to one or more mycotoxins was 79% with an annual exposure risk range of between 70 and 87% within the 5 years (Table 3, https://www.biomin.net/. Accessed on November 24, 2021). Other recent independent studies in the region have also revealed high incidence of mycotoxins in food and feed in the region has increased exposure to both humans and animals, with negative health, physical development and nutritional implications

**Table 1**
Mexico and Central America main diet crops consumed, processing method and aflatoxin regulations.

| Country | Crop | Amount consumed (kg/person/year)[a] | Common food processing methods[b] | $AFB_1$ limits/regulations | Reference |
|---|---|---|---|---|---|
| Guatemala | Maize | 87.25 | Nixtamalization, roasting, boiling | 20 μg/kg in maize grain/COGUANOR NGO 43 047 | COGUANOR (1982) |
| | Beans | 12.12 | Boiling | 20 μg/kg/FAO | FAO (2004) |
| | Rice | 5.66 | Boiling | | |
| Mexico | Maize | 116.34 | Nixtamalization | 20 μg/kg in maize grains/NMX-FF-034-SCFI 1995; NMX-FF-034/1-SCFI-2002; NMX-FF-034/2–2003; NOM-188-SSA1-2002<br>12 μg/kg in maize flour, tortilla and other nixtamalized products/NOM-247-SSA1-2008 | NMX-FF-034-1995 (1995); NMX-FF-034/1-SCFI-2002 (2002); NMX-FF-034/2-SCFI-2003 (2003); Norma Oficial Mexicana NOM-188-SSA1-2002 (2002); Normal Oficial Mexicana NOM-247-SSA1-2008 (2008) |
| | Beans | 10.38 | Boiling | No specific information found | |
| | Chili (Fresh and dried) | 18.4 | None (consumed fresh), roasting, drying | 30 μg/kg (proposed by CODEX Alimentarius Commission)[6] | |
| | Rice | 5.64 | Boiling | 20 μg/kg/NOM-188-SSA1-2002 | |
| Costa Rica | Maize | 10.77 | Nixtamalization | 20 μg/kg/Decreto 27980-S based on Codex Alimentarius[7] | Decreto Ejecutivo: 27980 (1999) |
| | Beans | 10.08 | Boiling | 20 μg/kg/Decreto 27980-S based on Codex Alimentarius[7] | |
| | Rice | 45.69 | Boiling | 20 μg/kg/Decreto 27980-S based on Codex Alimentarius[7] | |
| El Salvador | Maize | 70.03 | Nixtamalization | 20 μg/kg/FAO | FAO (2004) |
| | Beans | 17.32 | Boiling | 20 μg/kg/FAO | |
| | Rice | 10.53 | Boiling | 20 μg/kg/FAO | |
| Honduras | Maize | 77.96 | Nixtamalization | 1 μg/kg/FAO | FAO (2004) |
| | Beans | 12.05 | Boiling | 1 μg/kg/FAO | |
| | Rice | 14.42 | Boiling | No specific information found | |
| Nicaragua | Maize | 68.5 | Nixtamalization | No specific information found | |
| | Beans | 21.4 | Boiling | No specific information found | |
| | Rice | 43.3 | Boiling | No specific information found | |
| Panama | Maize | 25.5 | Dehulled and pre-cooked | No specific information found | |
| | Beans | 1.8 | Boiling | No specific information found | |
| | Rice | 66.4 | Boiling | 0 μg/kg/DGNTI-COPANIT-75-2002/DGNTI-COPANIT-74-2003 | DGNTI-COPANIT-75-2002 (2002); DGNTI-COPANIT-74-2003 (2003) |

[a] FAOSTAT (2021).
[b] INCAP (2007)

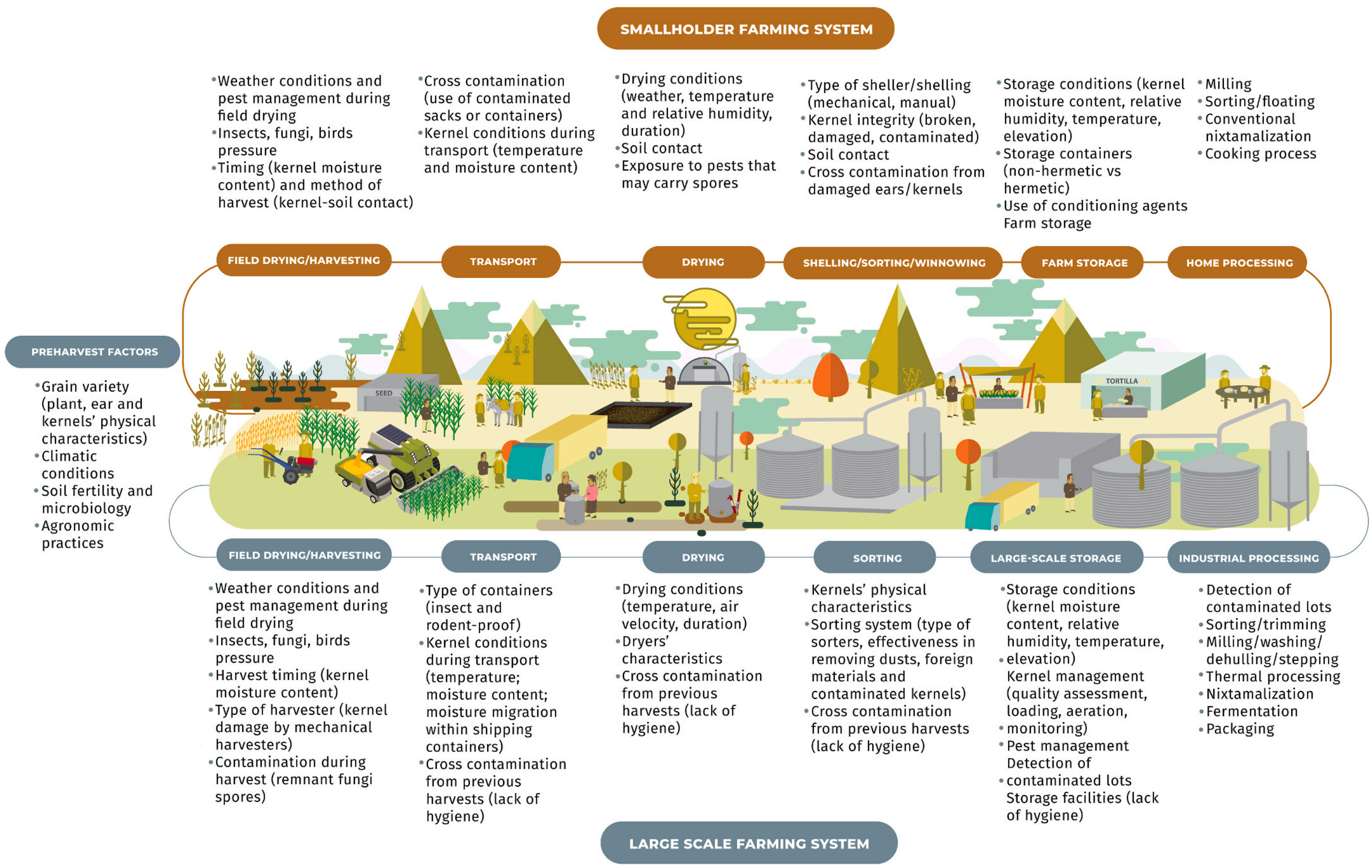


**Fig. 1.** Summary of actions to mitigate mycotoxin contamination in the maize value chain. Upper panel refer to actions in the smallholder farming system. Lower panel are action at the large-scale farming system.

**Table 2**
Fungi causing ear rot in maize, associated mycotoxin health effects and incidence in Mexico and Central America.

| Common name of fungi | Scientific name | Mycotoxin | Effects on human and animal health | Symptoms on maize ears | Coloration of fungi on infected maize kernels | Favorable conditions for ear infection | Mexican states with high incidence | CA countries with high incidence |
|---|---|---|---|---|---|---|---|---|
| *Aspergillus* | *Aspergillus flavus* *Aspergillus parasiticus* | Aflatoxin (B1, B2, G1, G2) | Carcinogenic, low immunological response can be lethal. | Start at tip of open | Powdery olive-green/ yellow | Drought and heat stress | Tamaulipas, Campeche, Sonora | Guatemala, Honduras, Panamá. |
| *Fusarium* | *Fusarium verticillioides* *Fusarium proliferatum* | Fumonisin (B1, B2) | Linked to esophagus cancer, pulmonary edema, immunosuppression, subfertility and poor nutrient retention. | Can be symptomless or cracked kernels or with white stripes | White | Poor soil fertility, Warm and dry weather | Sinaloa, Jalisco and Guanajuato | All CA countries |
| | | | | | Pink | Warm and humid weather | | |
| *Gibberella* | *Fusarium graminareum* | Zearalenone (ZEA) Deoxynivalenol (DON) | Follicular growth disorder, ovulation, atresia, hyper estrogenic syndromes, inhibition of protein synthesis, acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. | Start on the ear tip | Red | Cold and wet | Toluca and Estado de México | Honduras and Costa Rica |
| *Diplodia* | *Stenocarpella maydis* | Diploidiatoxin | Liver dysfunction, loss in body weight and feed intake. | Start on the ear base | Brown | Warm and wet | Chiapas and Guerrero | Belice and Honduras |
| *Penicillium* | *Penicillium. verrucosum* | Ochratoxin A | Necrotic renal tubules, periportal liver cells, immunosuppression, damage to the embryo and induction of cancer. | Start in ear at milky stage | Blue/green | Warm and humid | – | – |
| *Nigrospora* | *Nigrospora oryzae* | None reported | None | Start on the kernel tip | Black | High moisture in storage | – | – |

Sources: CABI, 2019; CIMMYT. 2004; Groopman et al., 2021; IARC. 2012; Martínez Padrón et al., 2013; Mendoza et al., 2018; Morales-Rodríguez et al., 2007.

**Table 3**
Summary of occurrence of aflatoxin, fumonisin, deoxynivalenol and zearalenone levels (μg/kg) in general cereals and finished product (2017, 2018 and 2021) and maize only (2019 and 2020) in Latin America as reported by Biomin.

| Mycotoxin | 2017[a] | | 2018[a] | | 2019[b] | | 2020[b] | | 2021[1c] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % of positive samples (total no. of samples analyzed) | Average mycotoxin levels to maximum level detected in μg/kg | % of positive samples (total no. of samples analyzed) | Average mycotoxin levels to maximum level detected in μg/kg | % of positive samples (total no. of samples analyzed) | Average mycotoxin levels to maximum level detected in μg/kg | % of positive samples (total no. of samples analyzed) | Average mycotoxin levels to maximum level detected in μg/kg | % of positive samples (total no. of samples analyzed) | Average mycotoxin levels to maximum level detected in μg/kg |
| **Aflatoxin** | 23 (N = 6943) | 8–1336 | 27 (N = 6023) | 8–402 | 21 (N = 4091) | 4–1264 | 12 (N = 3839) | 4–179 | 8 (N = 3861) | 7–2630 |
| **Fumonisin** | 75 (N = 5500) | 2992–218883 | 72 (N = 5465) | 2184–72100 | 90 (N = 3304) | 1700–170300 | 84 (N = 3577) | 1390–56000 | 73 (N = 3528) | 1489–64000 |
| **Deoxynivalenol** | 82 (N = 4849) | 919–12802 | 67(N = 5107) | 1008–24880 | 56 (N = 2608) | 340–5600 | 49 (N = 2568) | 350–6330 | 68 (N = 3380) | 581–4710 |
| **Zearalenone** | 51 (N = 6030) | 113–3553 | 48 (N = 5276) | 130–520 | 27 (N = 3472) | 53–2487 | 28 (N = 10511) | 60–4948 | 25 (N = 3467) | 82–3469 |
| **Total exposure risk** | **80** | | **70** | | **84** | | **87** | | **74** | |

Source: https://www.biomin.net/accessed on November 24, 2021.
[a] Denotes that mycotoxin data reported during the year are for all samples analyzed from the region including individual cereals like maize, wheat and oats as well as finished feed.
[b] Denotes that mycotoxin data reported during the year are for maize grain samples.
[c] Data only covers January to September months of the year 2021.

(Granados-Chinchilla et al., 2017; Groopman et al., 2021; Marimón Sibaja et al., 2021; Morales-Moo et al., 2020; Voth-Gaeddert et al., 2019, 2020). For instance, increased AFs exposure is linked to liver cancer and non-alcoholic cirrhosis incidence in the region. These two health conditions are among the top 10 causes of death in Mexico and Central America. The frequency of stomach and colorectal cancer is also increasing, while the incidence of growth stunting and micronutrient deficiency remains high despite concerted efforts to combat these (Alvarez-Banuelos, Carvajal-Moreno, Mendez-Ramirez, & Rojo-Callejas, 2015; Ponce-Garcia, Palacios-Rojas, Serna-Saldivar, & Garcia-Lara, 2021; J. W. Smith et al., 2017; Voth-Gaeddert et al., 2020; https://www.healthdata.org/results/country-profiles. Accessed on January 19, 2022). Additionally, intestinal microbiome has been associated with child stunting and AF exposure. Voth-Gaeddert et al. (2019) have recently added more evidence by assessing microbial differences for children in Guatemala, grouped by height, age, diarrhea and AF exposure. They found that children with a diet with more than 10 ng/kg of body weight/day had about 24 times higher odds of having a dysbiotic intestinal microbiome.

The intake of milk and its derivatives as well as an increase in animal protein sources in the region has exacerbated secondary exposure to various mycotoxins, especially AFs and FBs (Biomin, 2020; Carvajal-Moreno, Vargas-Ortiz, Hernández-Camarillo, Ruiz-Velasco, & Rojo-Callejas, 2019; Granados-Chinchilla et al., 2017; Marimón Sibaja et al., 2021). Dairy products are considered to be an important part of human diet in the region. However, when AFB1 is ingested by animals through contaminated feed, this mycotoxin undergoes biotransformation into aflatoxin M1 (AFM1) that is excreted in milk or eggs (Anfossi, Di Nardo, Giovannoli, Passini, & Baggiani, 2015; Wochner, Becker-Algeri, Colla, Badiale-Furlong, & Drunkler, 2017). AFM1 is potent but slightly less risky than AFB1. FB1 do not undergo any biotransformation they are therefore equally potent as when consumed through feed and food (Fink-Gremmels, 2008). This can therefore lead to double human exposure when primary dietary food sources and animal feeds are contaminated.

The mitigation of mycotoxin exposure is a multi-dimensional issue that goes from the field to the plate. Strategies to mitigate mycotoxins include pre- and postharvest actions as well as health, agricultural and trade regulations. During the pre-harvest period, common preventive measures include the use of appropriate crop varieties that are adapted to stress conditions or tolerant to the fungi, and appropriate agronomic management, including sowing date, adequate fertilization, crop rotation, insect control and the use of biological control agents like competitive non toxigenic strains (Mahuku et al., 2019; Martínez Padrón et al., 2013; Moral et al., 2020). Temperature and moisture control during storage and transportation can guarantee low water activity in kernels and are critical postharvest factors that can reduce mycotoxin accumulation. Processing and biotransformation are among other postharvest strategies used to mitigate consumer exposure to mycotoxins (Li et al., 2020; Villers, 2014).

This review discusses postharvest physical and processing strategies that can be adopted to reduce AF and FB incidence in the context of Mexico and Central America. This paper identifies and discusses some knowledge gaps that need to be addressed as part of several mycotoxin-related integrated actions that can help combat food safety issues, malnutrition and food insecurity in the region related to mycotoxins.

## 2. Occurrence of mycotoxins in Mexico and Central America

Grain production in Mexico and in Central America is characterized by a variety of agricultural systems, from smallholders growing landraces in rain-fed conditions mainly for self-consumption and local niche markets, to large-scale, market-oriented farming with high inputs and improved germplasm (Logrieco et al., 2021). Regardless of the farming system, one particular disease complex associated with maize deterioration and mycotoxin accumulation is generically referred to as maize ear rot fungi, which produce mycotoxins in symptomatic or asymptomatic grains (Ortiz et al., 2018). Ear rot pathogens can enter maize kernels through wounds caused by insects, through the silk, or systemically from the stalk (Jones, Payne, & Leonard, 1980; Munkvold, McGee, & Carlton, 1997; Murillo-Williams & Munkvold, 2008). The most common insects linked to *Aspergillus flavus* and *Fusarium verticillioides* infections are fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*) and maize weevil (*Sitophilus zeamais)* (Haidukowski, Farinós, & Patiño, 2021; Hutchison et al., 2010; Lussenhop & Wicklow, 1990).

AFs and FBs are the primary mycotoxins occurring in maize in Mexico and Central America (Table 2). *A. flavus*, *Aspergillus nomius* and *Aspergillus parasiticus* are the main producers of AFs, while *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium subglutinans* are the main producers of FBs (Morales-Rodríguez, Yañez-Morales, Silva-Rojas, García-de-Los-Santos, & Guzmán-de-Peña, 2007; Rosa Junior et al., 2019; Vaamonde, Patriarca, Fernández Pinto, Comerio, & Degrossi, 2003). *Fusarium graminearum* and *Fusarium culmorum* produce DON while *F. graminearum, F. culmorum, Fusarium cerealis* and *Fusarium equiseti* produce ZEN (Thapa, Horgan, White, & Walls, 2021). Other ear rot fungi include *Stenocarpella maydis*, *Nigrospora oryzae*, *Macrospora phaseoli* and *Penicillium* spp., which develop mainly when climate conditions are warm and humid (Table 2).

The symptoms of different ear rot fungi differ between species (Table 2). *Fusarium* ear rot symptoms are characterized by scattered tufts of mold on the ears ranging from white to pink and are accompanied by starburst patterns on the kernels. *Aspergillus* ear rot appears as green-yellow spores and is commonly observed after hot, dry weather in the post-pollination period. Drought-stressed maize plants such as those growing in non-irrigated fields and pivot corners are vulnerable to these pathogens (CIMMYT, 2004).

AFs were first identified in the 1960s and are mainly produced by *A. flavus*, *A. parasiticus* and *A. nomius*. The group consists of 18 chemical structures derived from difuranocoumarin with a coumarin nucleus-based bifuran group and a lactone ring or a pentanone ring (Richard, 2008). The most commonly occurring AFs are AFB1, B2, G1, G2, M1, and M2. *A. flavus* produces AFs, including aflatoxins type B (AFBs), which are considered to be the most carcinogenic and hepatocarcinogenic. The International Agency for Research on Cancer (IARC) groups AFB1, B2, G1, G2 and M1 as carcinogenic to humans and therefore classified as Group 1. AFM2 is considered to be potentially carcinogenic to humans hence classified as group 2B (IARC, 2002). FBs are a group of 28 structurally related mycotoxins produced by *F. verticillioides*, *F. proliferatum*, *F. nygamai*, *Alternaria alternata* f. sp. Lycopersici and *A. niger*. FBs are divided into four subtypes (Dall'Asta, Galaverna, et al., 2009; Upadhaya, Park, & Ha, 2010; Wan Norhasima, Abdulamir, Abu Bakar, Son, & Norhafniza, 2009). The most studied FB subtypes are B1, B2 and B3. FBs have been associated with esophageal cancer, pulmonary edema, immunosuppression, subfertility, increase in permeability to pathogens, and reduction in nutrient absorption at the intestinal barrier level; IARC classifies FBs as potentially carcinogenic group 2B carcinogens (IARC, 2002; Braun & Wink, 2018).

Further, mechanical damage, or biotic damage by insects or birds predisposes maize to colonization by fungi. Through 2020 regional surveys, FBs were found to occur at high levels in Mexico and Central America (Biomin, 2020). FB structure is similar to that of sphingolipids: sphinganine and sphingosine. They have a free amino group in C2 that inhibits ceramide synthase (sphinganine and sphingosine N-acyl-transferase), an important enzyme in the pathway of sphingolipid biosynthesis. The result is increased cytotoxicity (Sharma, He, & Sharma, 2004; Voss, Smith, & Haschek, 2007). Fumonisin B1 (FB1) is the most prevalent at 70% and its effects can be either acute or chronic in humans. Although the factors responsible for FB accumulation are not well understood, high FB levels in raw maize have been associated with heat and drought conditions with high humidity periods just before pollination. The optimum temperature for the growth of *F. verticillioides* and the production of FB1 is 25–30 °C (Bruns, 2003; Fandohan, Hell, Marasas, & Wingfield, 2003; F. M.; Liu, Chen, Fu, & Shih, 2005). The presence of *Fusarium graminearum* reduces the production of FBs by *F. verticillioides* (Fandohan et al., 2003). Further, nutrient deficiencies, especially of phosphorus, calcium and potassium, increase plant susceptibility to fungal attack. Hybrids grown out of their adaptive range and with no innate *Fusarium* resistance are also more susceptible to FB accumulation (Headrick & Pataky, 1991; Shelby, White, & Bauske, 1994).

As fungal AFs and FBs enter into the postharvest system, different factors may contribute to their accumulation or reduction, starting from drying in the field and harvesting and continuing through other postharvest activities including drying, sorting, and processing (Fig. 1). Whether it is a smallholder or large-scale farming system, conditions and operations that provoke grain contamination by fungi (for example lack of hygiene) and their subsequent development (for example moisture content and temperature during storage, may lead to the production of mycotoxins (Fig. 1). High temperatures in the food processing result in the formation of FB analogues such as N-carboxymethyl-fumonisin B1 or N-deoxy-D-fructose-1-yl-fumonisin B1. Compared to AFs, FBs are more prone to destruction through different food processing methods. However, the health effects of the resulting FB analogues are currently not well understood (Bryła, Roszko, Szymczyk, Jędrzejczak, & Obiedziński, 2016; Fandohan et al., 2005; Ponce-García, Serna-Saldivar, & Garcia-Lara, 2018).

As a result of the co-colonization of maize by the above complex of ear rot fungi, in addition to AFs and FBs, maize is also commonly predisposed to ochratoxins, trichothecenes and zearalenone. AFs and FBs co-occur in maize more frequently, posing an array of health risks to communities that consume maize as a staple diet (Smith, Madec, Coton, & Hymery, 2016). The potential for co-exposure of consumers to both AFs and FBs is likely to occur due to the Mesoamerican diet and the potential of *Aspergillus* and *Fusarium* to contaminate the staple, maize (Lee & Ryu, 2017). Thus, co-exposure can exacerbate the health and nutritional risks associated with the two mycotoxins, and concern is growing since the current regulations and risk assessment guidelines are mainly based on toxicity studies that focus on individual mycotoxins (Lee & Ryu, 2017; Torres et al., 2015). The health and nutritional impact of the interaction of AF and FB mycotoxins in food and diets must be a subject of study.

The maximum AFB1 limits for maize, beans and rice is 20 μg/kg in most countries in the region including Guatemala, Mexico, Costa Rica El Salvador and Nicaragua. Honduras is the most stringent in the region with maximum limits of 1 μg/kg for both maize and beans. Mexico is the only country in the region with maximum level for AF for chili at 30 μg/kg and with specific limits for grain and maize food products (Table 1). It must be noted that Mexico and the Central America countries do not have official regulations regarding FBs maximum limits. Even for the AFB1 levels there was reliance on secondary data sources like Codex Alimentaries Commission reports, FAO reports and U.S. Food and Drug Administration (FDA) reports to obtain mycotoxin guidelines for Central America countries (Table 1). Another concern is the high DON and ZEN level that call for urgent regulation. Clearly, the different maximum permissible mycotoxin levels in the region need to be documented and if possible harmonized by the respective regulatory government organizations for easy cross border trading.

## 3. Postharvest grain handling

### 3.1. Grain drying

In order to halt fungal development and the production of mycotoxins during grain transportation and storage, it is necessary to lower grain moisture after harvest. Large-scale, artificial drying is done for 7–10 days at 40 °C but the drying temperature can be over 100 °C depending on the end-use, for example for feed (Bala, 2016). A moisture content of 12–14% is recommended for a safe storage, which corresponds to an equilibrium relative humidity of 65% (Bradford et al., 2018). However, higher temperatures can affect seed germination and grain quality can deteriorate with high-speed and high-temperature drying by leading to a reduction in protein content, an increase in susceptibility to breakage, and an increase in hairline fractures (called stress cracks) in the endosperm. Subsequently, broken grains or grains with an increased number of stress cracks are more susceptible to insect and fungal damage during storage (Hawkins, Windham, & Williams, 2005).

Capacity for artificial drying is limited and the most common practice in Mexico and other Central American countries is field drying to a moisture level of between 14% and 18%. In a smallholder farming system, farmers use a variety of practices for field drying, including drying cobs and plants in their natural position, cutting the plants at maturation and leaving in the field in pyramidal-shaped stacks, or doubling the cobs downwards (a practice referred as “folding”, or “*doblado*” in Spanish) (Tigar, Key, Flores-s, & Vazquez-a, 1994). The same practices are reported in Guatemala where farmers complement them with drying in farmhouse attics (Mendoza, Sabillón, et al., 2017). In such a system, where farmers have little opportunity to monitor damage during field drying, infestation by fungi starts in the field and may be important depending on the environmental conditions, particularly in humid climates (Bradford et al., 2018). Moisture content at harvest such as that commonly found in the western highlands of Guatemala results in the development of a large microbial community, including a significant amount of *Fusarium* and *Aspergillus* mycotoxin-producing molds (Mendoza, Kok, Stratton, Bianchini, & Hallen-Adams, 2017). Farmers in Mexico also reported that additional atypical rains during drying can increase the development of toxigenic strains of fungi (González Regalado, Rivers, & Verhulst, 2017, pp. 42–48).

Some farmers use motorized shellers that can cause physical damage to grains and create entry points for fungal spores (Fandohan et al., 2003). Some of the common practices recommended for reducing the risk of AF and FB accumulation during postharvest activities include harvesting maize with the husk, sun drying on platforms, drying maize without the husk, and the immediate removal of damaged cobs prior to storage (Hell, Cardwell, & Poehling, 2003; Odjo, Burgueño, Rivers, & Verhulst, 2020). In addition, the promotion of low-cost drying solutions like the EasyDryM500 portable dryer developed in Kenya (Walker & Davies, 2017) or the BAU-STR dryer in Bangladesh have helped reduce the proliferation of fungi (Saha, Alam, Alam, Kalita, & Harvey, 2017) and could be affordable solutions for Mexico and Central American countries.

The Mexican norm for maize commercialization sets limits on the percentage of kernels broken or stress-cracked of between 2 and 7% (NMX-FF-034/2-SCFI-2003, 2003). These limits aim to ensure grain quality for optimal processing and grain safety. However, more regulations and guidance on grain quality in the region could help guarantee a reduction of mycotoxin levels and health impacts.

### 3.2. Grain sorting

Wounded, broken and damaged kernels or kernels with dead embryos accumulate more AFs and FBs, and this can worsen with high temperatures and moisture during the growing, harvesting and storage phases (Ortega-Beltran & Cotty, 2020); additionally, fine material, crop residuals and dust within grain batches may contain mycotoxins (Pascale et al., 2020). Healthy and contaminated kernels have shown significant differences in size, shape and especially density (Pascale et al., 2020). Hand- or mechanical cleaning operations targeting the elimination of defective kernels, especially those with off-coloration, germ- or endosperm heat damage, broken kernels, and kernels with visible fungal or insect damage are known to effectively reduce mycotoxin levels during storage (Fandohan et al., 2005; Pascale et al., 2020). In fact, these operations are promoted by health and trade regulatory agencies and policies (FAO (2004); FDA (2020), Codex Alimentarius, NMX-FF-034/1-SCFI-2002, 2002). Sieves, gravity separators, color sorters or electronic sorters can contribute to reducing from 40 to 95% of AFs and FBs by removing off-colored and low-density kernels that have been damaged by heat, insects, or fungi (Pascale et al., 2020).

At a household level, small farmers in Mexico and Central America that use maize for self-consumption clean the grain manually — a time-consuming task that does not guarantee a decrease in AF and FB exposure. This is because in most cases the discarded grain is given to backyard animals whose products are later consumed (Odjo et al., 2020). In addition, grain sorting might not be done systematically depending on the food-security context, and damaged grain could therefore be mixed with sound grain, as reported by Mendoza, Sabillón, et al. (2017) in Guatemala. In both large-scale, industrial farming and small-scale farming, the effect of grain cleaning varies depending on the level of contamination in the grain and on the percentage of contaminated grain removed (Pascale et al., 2020).

Grain integrity, including intact pericarps and embryos as well as kernel wax content, is reportedly fundamental to reducing fungal infection and AFs production (Chen, Brown, Damann, & Cleveland, 2004; Ortega-Beltran & Cotty, 2020). At the household level and during the wet-nixtamalization process at the industrial level, contaminated low-density and damaged maize kernels that are used for tortilla production in Mexico are commonly removed before nixtamalization or during the steeping stage of the process, because such kernels float in the nixtamalization liquid (Serna Saldivar & Rooney, 2015). Although flotation is, to some degree, effective in reducing mycotoxin contamination, a significant proportion of mycotoxins can be carried over into the next steps in the nixtamalization process (Matumba, Van Poucke, Njumbe Ediage, Jacobs, & De Saeger, 2015). A combination of different sorting methods can be effective, but the use of combined methods within the regional context remains to be investigated.

Ortega-Beltran and Cotty (2020) found lower AFs levels in ground kernels compared to wounded kernels, suggesting that storing flour could be more effective if insect or mechanical damage has occurred during crop growing or harvest. However, as they also point out, this would not be appropriate for consumers in Mexico and Central America as most of the maize is used for nixtamalization that uses whole kernels (Ortega-Beltran & Cotty, 2020; Palacios-Rojas et al., 2020). Additionally, storing flour could bring other challenges like limited shelf life due to increased rancidity (Goffman & Böhme, 2001).

### 3.3. Storage

In Mexico and Central America, smallholder farmers use a variety of containers to store their grain, including traditional wooden structures, metal containers and polypropylene sacks. But unfortunately, none of them sufficiently protect the grain against pest infestation (Giles & Leon, 1974; González Regalado et al., 2017, pp. 42–48; Mendoza, Sabillón, et al., 2017; Tigar et al., 1994). Around 40% of maize can be lost postharvest in Mexico’s tropical regions (García-Lara, García-Jaimes, & Ortíz-Islas, 2020; Odjo et al., 2020). Fungal contamination and mycotoxin development account for part of these losses. Some farmers may choose to treat their grain with insecticides, including aluminum phosphine tablets and deodorized malathion, two products authorized in Mexico, at doses between 4 and 6 tablets of aluminum phosphide per ton of grain and 1% weight-by-weight of deodorized malathion (Odjo et al., 2020). However, there are farmers that treat their grain at higher doses (1 tablet of aluminum phosphide per 50 kg of grain) (González Regalado et al., 2017, pp. 42–48) and little is known about the potential effect of the use of these chemicals on the health. Cases of intoxication by the use of insecticides are reported, but their use in storage has to be discouraged, particularly in smallholder conditions (Bernardino Hernández, Torres Aguilar, Sánchez Cruz, Reyes Velasco, & Zapién Martínez, 2019; González Regalado et al., 2017, pp. 42–48; Odjo et al., 2020; Villa-Manzano, Zamora-López, Huerta-Viera, Vázquez-Solís, & Flores-Robles, 2019).

Few farmers use currently available hermetic storage technologies like hermetic plastic bags and hermetic metal silos (García-Lara et al., 2020; Odjo et al., 2020; Villers, 2014). *Aspergillus* species and fungi in general are aerobic, and therefore their development and the synthesis of mycotoxins cease when the fungi are exposed to oxygen levels lower than 0.025% of environmental levels. Oxygen can be considered a critical element for the growth, but not for the survival of these fungi. Likewise, $CO_2$ in concentrations of 20% is known to halt the germination of spores and suppress the synthesis of AFs at levels greater than 10% (CAST, 2003). Thus, increased deployment of and training in the use of high-quality hermetic storage technologies in the region will benefit AFs and FBs mitigation strategies (García-Lara et al., 2020; Odjo et al., 2020).

Regarding large-scale storage systems, data on the management of warehouses and the occurrence of mycotoxin contamination in Mexico and Central America is scarce. A study conducted in Mexico has, however, reported that most grain warehouses have minimal or no equipment, which may have a significant impact on the standards of quality management including mycotoxin contamination (Ortiz Rosales, Ramírez Abarca, González Elías, & Velázquez Monter, 2015). The training and application of a quality management system such as the SLAM (Sanitation, Loading, Aeration, and Monitoring) strategy (Mason & Woloshuk, 2010) or the HACCP (Hazard Analysis and Critical Control Points) approach can help in minimizing mycotoxin occurrence in these warehouses. Most of these warehouses rely on aluminum phosphine tablets for control, but postharvest pests resistant to phosphine have been reported in the region (Afful, Elliott, Nayak, & Phillips, 2018). The use of ozone may be an alternative and has been successfully used to reduce AFs elsewhere. Maeba, Takamoto, Kamimura, and Miura (1988) determined the inactivation of the mutagenic activities of AFs via ozonolysis. However, their results showed that $AFB_2$ and $AFG_2$ were more resistant to degradation (34.3 mg/L over 50–60 min) compared to $AFB_1$ and $AFG_1$ (1.1 mg/L over 5 min). Luo et al. (2014) determined that the AFB1 degradation rate increased with ozone concentration and the maize kernels' treatment time. Kernel moisture content had a negative correlation with the degradation rate. Ozone application for at least 40 min degraded fungi and reduced $AFB_1$ by up to 88%. To our knowledge, the use of ozone-treated maize in Mexico or Central America countries is very limited but it might be an alternative for the future.

### 3.4. *Grain conditioning agents, adsorbents and other food and feed additives*

Farmers in Mexico and Central America may also use grain-conditioning agents, other than chemical insecticides, that potentially have an insecticidal effect and are used in combination with polypropylene bags or any other non-hermetic containers. For example, smallholder farmers in this region may use inert dusts as conditioning agents to control pests. This category of adjuvants includes diatomeceaous earth, limestone, clays, zeolites, ash, sand and is considered as Generally Regarded As Safe (GRAS) additive in food and feed. by the U. S. Food and Drug Administration (Subramanyam & Roesli, 2000). The use of lime during storage was reported by the ancient Aztecs and is still used today in Mexico and Central America (Golob, 1997; González Regalado et al., 2017, pp. 42–48). While inert dusts seem to be a non-toxic solution to the problem of insect control, their effectiveness in minimizing losses, which depends on relative humidity and temperature, can be reduced in tropical conditions (Odjo et al., 2020). Data on the use of lime on AFs and FBs levels are scarce, however. But the effects of similar conditioning agents on mycotoxins have been investigated elsewhere. Some studies have shown that the synthetic zeolite NaA (sodium aluminum silicate) is not effective in binding aflatoxin B1 (AFB1) in feed in comparison with bentonites (Vekiru et al., 2015). The mechanism of the interaction between hydrated sodium-calcium aluminosilicate clay and AFB1 is the chelation of the toxin carbonyl groups with metal ions or metal surface sites at a low pH. Bentonites are widely accepted, low-cost binding materials with a high specific surface area for reducing the toxic effects of AFB1. Moreover, they improve the palatability and durability of supplemented feed. Bentonites containing *cis*-vacant smectite, C2 *trans*-vacant smectite, and clinoptilolite containing zeolite Z08 have been successfully tested. However, the effectiveness of bentonites depends on the chemical batch and source of extraction (geological deposit), dose, and AF loads in the feed to be treated (Magnoli et al., 2008; Vekiru et al., 2015). A clinical trial tested the oral ingestion of calcium montmorillonite clay capsules or the same adsorbent supplemented directly to the diet. The study concluded that the clay effectively reduced AFs without detrimental effect in terms of palatability and food acceptability. However, further studies are needed to determine if these adsorbents cause side effects, especially in essential mineral bioavailability (Awuor et al., 2017). Despite the high prevalence of AF-contaminated foods in Mexico and Central American countries, the use of adsorbents in foods is currently prohibited because they bind essential micronutrients. The supplementation of nixtamalized *masa* — a coarse-ground, cohesive corn dough — with adsorbents to selectively bind AFs in tortillas and related products has not been the subject of research.

Mexico and Central America are recognized for their biological diversity, and pre-Columbian civilizations used aromatic and medicinal plants in their daily activities. Some of these plants have been used as food additives due to the essential oils they contain that have insecticidal and repellent effects against insects, antimicrobial effects against fungi, and anti-aflatoxigenic properties (Palma-Tenango, Miguel-Chávez, & Soto-Hernández, 2017; Pöll, 2005). Essential oils from aromatic plants are widely accepted because of their relatively low adverse effects (rare allergic reactions), high volatility and biodegradability. Rangel-Fajardo, Tucuch-Haas, Burgos-Díaz, Gómez-Montiel, & Basto-Barbudo, 2020 and Hernández-Cruz et al. (2019) have demonstrated that ground dry leaves of the Mexican tea plant *Dysphania ambrosioides*, and essentials oils from *Porophyllum linaria* can control damage caused by the maize weevil due to their terpene compounds, which may in turn limit fungal infestation. Juárez, Hernández, Bach, Sánchez-Arreola, and Bach (2015) reported the antifungal activity of essential oils extracted from *Agastache Mexicana* ssp. *xolocotziana* against fungal strains including *A. flavus* isolated from stored grain. Extracts from *Buddleja perfoliate* and *Pelargonium graveolens* have also shown the same potential (Juárez, Bach, Sánchez-Arreola, Bach, & Hernández, 2016). However, data on the use of these aromatic plants by smallholder farmers are scare, and a diagnostic made in Mexico has shown that only 1% of farmers surveyed use this strategy to protect their grain during storage (González Regalado et al., 2017, pp. 42–48). Even though recent research has shown that the use of these plant extracts has no significant effect on product quality (Juárez, Bach, Bárcenas-Pozos, & Hernández, 2021), their suitability for generalized use has yet to be proven, given the potential effects on sensory quality of the food products, risk to the products' forms, and the method's practicality at large scale.

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## 4. Grain processing

During food processing, mycotoxins can be redistributed, degraded, modified, bind or be released from the food matrixes. Mycotoxin content can also be altered by dilution or concentration effects due to moisture changes or when different ingredients are mixed (Schaarschmidt & Fauhl-Hassek, 2021). Thus, the stability of mycotoxins is affected by moisture, heating regime, type of cereal/food matrix, pH levels and even external forces like pressure or shear forces. Meal composition can also contribute to lowering the bioaccessibility and bioavailability of mycotoxins (Lin, Hu, Zhang, Xia, & Zhang, 2019). AF are more stable than FBs. FB are heat stable up to 100 °C, although significant decrease of detectable FBs has been reported. Chemical degradation of FBs occurs via Maillard-type reactions at high temperature or hydrolysis of FBs also occurs in the presence of alkali (Ponce-García et al., 2018). Moreover, in the past decade or so, the presence of FBs bound to proteins or other food components as well as FBs physical entrapment into the structure of macromolecular components like starch and proteins have been demonstrated, as well as its implication in the accuracy of FBs monitoring in food products (Dall' Asta et al., 2009).

Fig. 2 depicts the most common processes used in Mexico and Central America to transform maize kernels into dishes. Thermal processes are the most common ones, including both dry heating (baking, roasting, popping) and wet-heating (lime-cooking and the subsequent baking, frying, steaming processes). Ranges of reductions in AF and FB areas are also included in this figure. These ranges are based on several reports analyzed by Schaarschmidt and Fauhl-Hassek (2021) and specific references for Mexican and Central American food products described in more detailed below.

### 4.1. Baking, roasting and popping

Baked maize products in the region include maize bread, a diverse array of maize cookies (*semitas*, *coricos*, *pemoles*, *gorditas*, *polvorones totopos*, *tlayudas*, etc.) and flat bread prepared with decorticated maize (*arepas*, consumed in Panama). Some of these products are consumed sporadically, during special festivities, at a specific time of the year, or in specific towns/areas (Fernández Suárez, Morales Chávez, & Gálvez Mariscal, 2013; Guzzon et al., 2021). The average reduction in AFs during baking ranges from 15 to 50% and to about 70% for FBs (Seenappa & Nyagahungu, 1982); the degree of variation depends on the temperature and length of baking, as well as the presence of other ingredients. To our knowledge, however, there is no report on concentrations of AFs or FBs in baked maize products in Mexico and Central American countries.

Maize kernels are commonly toasted, milled, and mixed with sugar cane and cinnamon or other flavors. This flour is consumed directly or diluted in water or milk (*atole*) (Guzzon et al., 2021). Roasting can have a stronger effect on the degradation of mycotoxins, because normally temperatures are higher, at a higher surface to volume ratio and

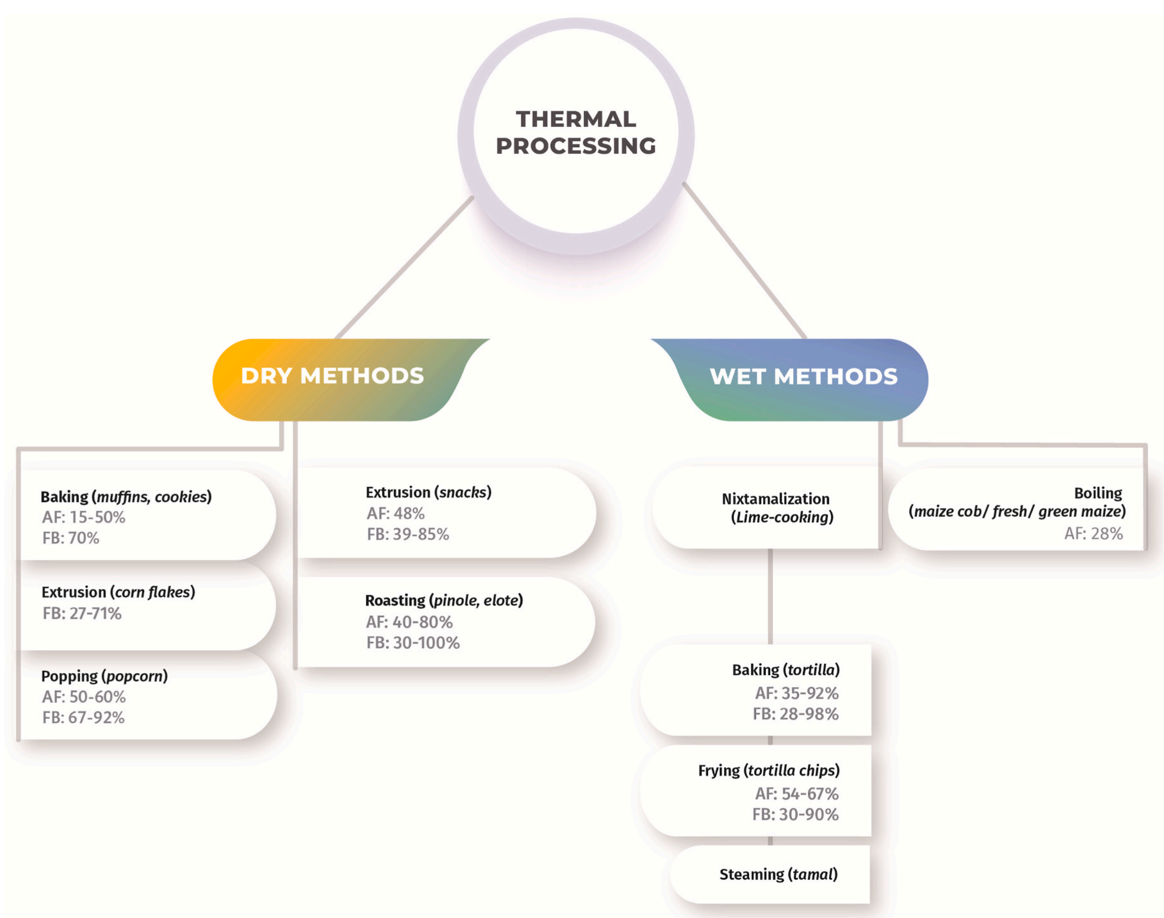


**Fig. 2.** Percentages of reported losses of AFs and FBs in food products derived from common maize processing methods used in Mexico and Central America.

therefore a higher internal temperature. About 30–100% reduction in FBs and 40–80% reduction in AFs have been found in dry heating impact studies on mycotoxins (Schaarschmidt & Fauhl-Hassek, 2021). Méndez-Albores, De Jesús-Flores et al. (2004)monitored the fate of AFs in spiked maize kernels toasted and boiled to prepare *atole* and found that the toasting led to about 30% reduction in $AFB_1$, while a further moderate reduction occurred during the boiling step.

Popping maize kernels by heating at 220–230 °C leads to a reduction of around 50% in $AFB_1$, independently of whether the kernels are spiked, inoculated with the fungi, or naturally contaminated (Rehana & Basappa, 1990). Recent surveillance of popcorn maize samples collected in 30 different places in the city of Veracruz found that about 47% of the samples had significant levels of AFs, indicating that the popping process was not enough to remove the AFs, and that it is therefore important to ensure that the grain to be processed is clean (Morales-Moo et al., 2020). D'Ovidio, Trucksess, Devries, and Bean (2007) analyzed FBs in popcorn from the optical reject stream that contained about 1 ppm of FBs. After popping in a microwave oven, a 67–92% reduction in the FBs was observed.

### 4.2. Alkaline-cooking or the nixtamalization process

Nixtamalization or alkaline-cooking is a Mesoamerican ancient technique for transforming maize into hundreds of different food dishes (Palacios-Rojas et al., 2020). The traditional and industrial methods used today consist mainly of cooking maize kernels in a lime solution (calcium hydroxide) for about 30–40 min, followed by overnight steeping for 8–16 h. The lime-cooked grains are washed to remove excess lime and then stone-ground to transform them into masa. The resulting masa is formed into tortilla discs that are baked in continuous gas-fired ovens, although it is thought that about 300 other food products are derived from such dough (Palacios-Rojas et al., 2020). After lime-cooking, other processes like baking, frying and steaming are used to prepare the final dishes (Fernández Suárez et al., 2013). In Fig. 2 the incidence on the AFs and FBs in some of the most popular nixtamalized food products is summarized. Tortillas are considered the most common staple food in Mexico and Guatemala. They are also highly consumed in El Salvador, Honduras and Costa Rica (Table 1). On average, Mexicans in rural and urban areas consume 79.5 and 56.7 kg of tortillas each year, respectively (CEDRSSA, 2014). These amounts are equivalent to seven and five tortillas daily, which supply 477 and 340 kcal, respectively. In addition to tortillas, several other food products including *sopes, tlacoyos, salbutes, chalupas*, etc are prepared by baking the nixtamalized dough. Nixtamalized dough is also baked and fried to produce chips or *totopos, chilaquiles,* etc., or it can be steamed to make *tamales*, a very common breakfast dish in Mexico and Central American countries. *Atoles,* a popular drink, also given to children as weaning food, can be prepared by diluting the nixtamalized dough with water or milk (Fernández Suárez et al., 2013; Guzzon et al., 2021; Martorell, 2020).

Cooking time and temperature, lime concentration, steeping time, and intensity of the washing step vary from household to household, within each country, and among the Mesoamerican countries. Industrial production of nixtamalized dry milling flour is accomplished by the sorting and removal of damaged kernels, lime-cooking, nixtamal washing, grinding, drying, sieving, regrinding coarse particles, resieving, classifying and blending to meet specific requirements regarding particle size distribution, water absorption and pH (Serna Saldívar & Perez Carillo, 2016).

In addition to the nutritional benefits of the nixtamalization process due to the increased intake of calcium, increased niacin bioavailability and fiber intake, and reduction in phytate (Escalante-Aburto et al., 2020; Palacios-Rojas et al., 2020), nixtamalization contributes to a reduction in FBs and AFs, mainly due to the physical loss of the pericarp into the residual cooking- and steeping-water known as *nejayote* (cooking liquid). Lime is known to hydrolyze fiber components in maize and enhance pericarp detachment from the rest of the kernel (Serna Saldivar & Rooney, 2015). The hydrolyzed pericarp, the partial removal of the germ and the leaching of FBs and AFs into the cooking liquid are the main mechanisms responsible for the significant reduction of these harmful metabolites (Odukoya et al., 2021; Schaarschmidt & Fauhl-Hassek, 2019; Serna Saldivar & Rooney, 2015). During the nixtamalization, the alkaline treatment remove two tricarballylic acid side chains from the 20-carbon backbone of FB1 resulting in the formation of hydrolyzed fumonisin B1 (HFB1) (Hartl & Humpf, 1999). Dombrink-Kurtzman and Dvorak (1999) and Voss, Poling, Meredith, Bacon, and Saunders (2001) described the fate of $FB_1$ and hydrolyzed $FB_1$ during the nixtamalization and commercial processing of fried tortilla chips, respectively, and found that more the 80% of those mycotoxins were lost in the process, especially during the nixtamalization and rinsing steps. Although HFB1 has greater cytotoxicity than FB1, it is less toxic in vivo (Hopmans & Murphy, 1993; Park, Scott, Lau, & Lewis, 2004). Voss, Ryu, Jackson, Riley, and Gelineau-Van Waes (2017) monitored the effectiveness of extrusion and nixtamalization methods by rat-feeding bioassays and found that neither $FB_1$ nor the $HFB_1$ caused neural tube effects in experimental mice and suggested that extrusion and nixtamalization reduce the potential toxicity of FB1 contaminated maize.

AFs have also been found to be reduced through nixtamalization: the reported $AFB_1$ reductions range from 28% to 98%, depending on the level of AF contamination, the nixtamalization process used, and the degree to which the nixtamalized kernels were contaminated prior to the processing (Fig. 2). A steeping time longer than 6h, a calcium hydroxide concentration higher than 1%, and an intense grain-washing step will impact the final content of mycotoxins in the food products (Guzmán-De-Peña, 2010; Palacios-Rojas, unpublished data). In the nixtamalization process, the grain cooking at 90 °C and the very high pH (12–14), the degradation of AFs is induced by the hydrolytic cleavage of the lactone ring of AFs, which is followed by decarboxylation (Schaarschmidt & Fauhl-Hassek, 2019). However, if the processing conditions are not optimal to induce the decarboxylation, the cleavage of the lactone ring can be reversed at the lower pH during the human digestion process (Méndez-Albores, Arámbula-Villa et al., 2004; Moctezuma-Zárate et al., 2015; Price & Jorgensen, 1985). The fact that AF adducts have been found in tortilla consumers might indicate that reactivation of the AF ring is taking place, or that not all AFs were fully detoxified during the nixtamalization process, Their effect and accumulation can also be exacerbated by the amount of tortillas and of other products potentially contaminated with AFs that are commonly consumed with nixtamalized tortillas as part of the daily diet, including beans, chili peppers or rice (Table 1) (Kroker-Lobos et al., 2019).

Alternative methods to the traditional nixtamalization have been developed and studied, particularly because of the drawbacks of traditional nixtamalization, such as long steeping times leading to more energy expenditure, and the high production of cooking liquids to contain the polluting residues (Escalante-Aburto et al., 2020). More ecologically-friendly methods, such as microwaving and the use of other salts instead of calcium hydroxide, have also been tested for their impact on AFs. In tortillas processed with traditional nixtamalization, the total AF degradation rate was 92%. In tortillas created through the ecological process, degradation rates were between 61% and 78% (Méndez-Albores, Villa, et al., 2004). The effects of the calcium sources on the fate of AFs in nixtamalized foods should be explored further. For example, some alternative nixtamalization processes use sources other than calcium hydroxide (lime), such as wood ash, $CaCO_3$, $CaSO_4$, $CaCl_2$, and $C_6H_{10}CaO_6$ (Santiago-Ramos et al., 2018). Odukoya et al. (2021) found a significant reduction of FBs in nixtamalized maize and sorghum when using wood ash, calcium hydroxide, sodium hydroxide and potassium hydroxide; however, tortilla quality and sensory tests might be important when using different lime sources, especially in a Mesoamerican context where the tortilla is part of the culture and consumers can be very knowledgeable about how a nixtamalized tortilla should taste.

Nixtamalized extruded maize is used at mid- and large-scale to produce chips and snacks. Maize-based breakfast cereals are also produced by extrusion. This cooking method involves a mixture of ingredients (maize, water, lime, sugars, and/or additives), cooking under pressure, and shear forces due to the rotating screw and extrusion at very high pressure for moisture reduction and shaping. Schaarschmidt and Fauhl-Hassek (2021) have summarized several reports on the effect of extrusion conditions on the mycotoxin levels. In general, extrusion has a high impact on mycotoxin content, but it can vary depending on which ingredients are included, the extrusion conditions, and the type of mycotoxin (Martinez & Monsalve, 1989; Elias-Orozco, Castellanos-Nava, Gaytán-Martínez, Figueroa-Cárdenas, & Loarca-Piña, 2002) (Fig. 2). The inactivation of $AFB_1$, $AFB_1$, and $AFB_1$-dihydrodiol in the extrusion process using lime together with hydrogen peroxide showed higher elimination of $AFB_1$ than treatments with lime or hydrogen peroxide alone. The extrusion process with 0.3% of lime and 1.5% of hydrogen peroxide was the most effective at detoxifying maize tortillas. A high level of these reagents negatively affected sensory characteristics such as the taste and aroma of tortillas compared with those produced by traditional nixtamalization (Elias-Orozco et al., 2002). Although hydrogen peroxide is a chemical oxidant with high potential to reduce AFs, it is not allowed in EU countries, but it can be used in other countries at very low levels. The FDA allows the use of food-grade reagent as an anti-microbial and with a maximum limit of 0.001% w/w (Food & Drug Administration (FDA), 2020; Normal Oficial Mexicana NOM-247-SSA1-2008, 2008).

The reduction of FBs in extruded maize has been shown in different studies and can vary from 30 to 90%. As for the AFs, the FBs reduction depends on level of initial contamination, moisture, temperature, screw speed and extrusion die (Castelo, Jackson, Hanna, Reynolds, & Bullerman, 2001; Cortez-Rocha, Trigo-Stockli, Wetzel, & Reed, 2002; Scudamore, Guy, Kelleher, & MacDonald, 2008). In general, lower moisture led to higher reductions of FB and HFB. Katta, Jackson, Sumner, Hanna, and Bullerman (1999) and De Girolamo, Solfrizzo, and Visconti (2001) have point out, however, that extrusion conditions that led to product with acceptable color and expansion not necessarily led to high reductions of FB1. During the applied direct extrusion is where most of the FBs reduction occurs; while in pellet extrusion and frying pellets not changes or very low reduction can occur (Scudamore et al., 2008). In addition to the technical extrusion parameters, the type and amount of ingredients added in the process, like salt, glucose or other sugars, can have positive impact in FBs reduction (Bullerman et al., 2008; Jackson et al., 2011; Scudamore et al., 2008). N-acyl FBs derivatives can be formed when glucose is added, including *N*-(carboxymethyl)-fumonisin B1 (NCM-FB1) and *N*-(1-deoxy-D-fructos-1-yl)-fumonisin B1 (NDF-FB1) (Bullerman et al., 2008; Jackson et al., 2011; Ponce-García et al., 2018). Seefelder, Hartl, and Humpf (2001) reported very low concentrations of NCM-FB1 in extruded commercial products from Germany and point out the low risk due also to the low toxicity of this FB derivative. To our knowledge, detailed monitoring of FBs derivatives is not a common practice, especially in the large informal industry of extruded products that can be found in Mexico and Central American countries.

The so called "hidden fumonisins" refer to FBs to noncovalently matrix-bound derivatives. The use of alkaline hydrolysis procedures has contributed to the determination of hidden FB1 in maize (Dall'Asta, Falavigna, Galaverna, Dossena, & Marchelli, 2010). A very small survey including nixtamalized flour and tortilla chips revealed the co-occurrence of FBs with partially hydrolyzed FB (PHFB) and HFB (De Girolamo, Lattanzio, Schena, Visconti, & Pascale, 2014). De Girolamo, Lattanzio, Schena, Visconti, and Pascale (2016) have also shown the that nixtamalization reduce the number of FBs and PHFBs, converting them to HFBs. The alkaline process also made available matrix-associated FBs as it was revealed by the increase of total amount measured in the raw maize. Although nixtamalization have advantages in providing safer products in terms of FB contamination, studies at higher/real scale will be beneficial. In addition, appropriate monitoring of the hydrolyzed forms of FBs as well as other hidden mycotoxins like zein-bound zearalenone is important to further assess the nutritional and safety properties of this technology and measure the mycotoxin exposure risk of the consumers (Tan, Zhou, Guo, Zhang, & Ma, 2021; De Girolamo et al., 2016). The development and deployment of cost-effective methodologies and availability of proper standards could facilitate such monitoring at higher scale in the region.

## 5. Co-exposure to aflatoxins and fumonisins in Mexico and Central America

The high consumption of tortillas and other nixtamalized, maize-derived products has led to high exposure to AFs and other mycotoxins in the region. As presented in Table 1, in Mexico and the Central American region, regulations for $AFB_1$ are different among countries: regulations are limited to raw grain; they do not exist; or they are not easily accessed or found. In Mexico, the regulatory limit for maize tortillas is 12 μg/kg of total AFs (Norma Oficial Mexicana NOM-187-SSA1/SCFI-2002, 2002). In Guatemala, El Salvador and Costa Rica, the limit is 20 μg/kg in maize grain, but the regulations do not clearly specify the levels for nixtamalized tortillas (Table 1) (COGUANOR, 1982; FAO, 2004; Food & Drug Administration (FDA), 2020). Unless accepted AF levels in crops as well as food products are revised and standardized and regulations are enforced, international development efforts to improve food security, nutrition and health are at risk (Lizárraga-Paulín, Miranda-Castro, Moreno-Martinez, Torres-Pacheco, & Lara-Sagahón, 2013). Equally important is the regulation and monitoring of FB and HFB levels, not only in the maize value chain, but also in other relevant dietary crops in the region. Table 4 summarizes published surveillance studies of AFs and FBs in common maize products in Mexico and Central American countries. Biological and methodological variability (from sampling to analytical recovering, matrix composition, moisture) are challenges to consider when comparing data and drawing conclusions on mycotoxin exposure (Schaarschmidt & Fauhl-Hassek, 2021). Nevertheless, taking together the mycotoxin surveillance in food products and the clinical studies, the risk of exposure to AFs and FBs by the Mexican and Central America populations is evident. In some of the studies, the levels of AFs and/or FBs seem low or even within the ranges accepted by the regulations. However, it is important to consider that exposure can be exacerbated by the quantity of contaminated food that a person is consuming. Although nixtamalization and other food processes used in the region could have an impact on reducing AFs and FBs, nothing that is done at postharvest level, including appropriate food-processing conditions, will ensure mycotoxin elimination if grain is highly contaminated in the field.

Surveys and clinical studies in the region, mainly in Mexico and Guatemala, have shown the presence of FB1 in human urinary samples (Riley et al., 2012; Torres et al., 2014, 2015; Yun et al., 2008). In addition, and as summarized and highlighted by Ponce-Garcia et al. (2021), there is increased evidence of AF abducts found in different populations during clinical studies in the region, as well as an increase in diseases and deaths where the causes are associated with AF exposure, including hepatocellular carcinoma, cervical cancer, child stunting and dysbiotic intestinal microbiome (Díaz de León-Martínez et al., 2021; Ponce-Garcia et al., 2021; Voth-Gaeddert et al., 2019).

Co-occurrence of FBs and AFs is of great concern given the potential of FB1 to modulate AFB1 hepatoxicity (Torres et al., 2015). Co-presence of FBs and AFs in the same grain has been documented from field to processed food, and co-exposure has been highlighted in clinical studies. Mendoza et al. (2018) reported the presence of AFs and FBs in 50% of the grain samples collected from 25 farmers in Huehuetenango department in Guatemala, and in 2015, Torres et al. reported FBs and AFs in 640 samples collected in 22 departments of Guatemala. Trucksess, Dombrink-Kurtzman, Tournas, and White (2002) found both FBs and AFs in *incaparina*, a mixture of maize and cottonseed flour supplemented with vitamins and minerals and used as a high-protein food supplement,

**Table 4**
Surveillance studies on occurrence of aflatoxins and fumonisins in maize products in Mexico and Central America. *NR: not reported.*

| Maize product | Process | Country of study | Fumonisins | Aflatoxins | Reference |
|---|---|---|---|---|---|
| Incaparina | Milling | Guatemala | n = 8<br>100% with $FB_1$, $FB_2$, $FB_3$<br>0.2–2.2 μg/g | n = 8<br>100% with $AFB_1$, <214 μg/kg<br>100% with $AFB_2$, <32 μg/kg | Trucksess et al. (2002) |
| Pozol | Nixtamalization + boiling | Mexico | NR | n = 111<br>17% with $AFB_1$ and $AFB_2$<br><20 μg/kg | Méndez-Albores, De Jesús-Flores et al. (2004) |
| Popcorn | Popping | Mexico | NR | n = 30<br>40% AFs<br>0–26 μg/kg $AFB_1$<br>0–51 μg/kg $AFB_2$<br>0–46 μg/kg $AFG_1$<br>0–28 μg/kg $AFG_2$ | Morales-Moo et al. (2020) |
| Tortilla | Nixtamalization + baking | Mexico | NR | n = 37<br>15% with $AFB_1$<br>>20 μg/kg | Palacios-Rojas ( unpublished data) |
| Tortilla | Nixtamalization + baking | Mexico | NR | n = 50<br>4% with $AFB_1$<br>>12 μg/kg | Rodríguez-Aguilar et al. (2020) |
| Tortilla | Nixtamalization + baking | Mexico | n = 64<br>98% with $FB_1$ and $FB_2$; $FB_1$ 9–1589 ppm; $FB_2$ 24–524 μg/g | NR | Gilbert Sandoval et al. (2019) |
| Tortilla | Nixtamalization + baking | Mexico | n = 120<br>90% with $FB_1$<br><526 μg/g | n = 120<br>71% with $AFB_1$<br>0–22 μg/kg | Wall-Martínez et al. (2019) |
| Tortilla | Nixtamalization + baking | Mexico | n = 88<br>4% $AFB_1$<br>>1 μg/g | NR | Gilbert Sandoval et al. (2019) |
| Tortilla | Nixtamalization + baking | Mexico | NR | n = 171<br>50% with $AFB_1$<br>0–287 μg/kg | Zuki-Orozco, Batres-Esquivel, Ortiz-Pérez, Juárez-Flores, and Díaz-Barriga (2018) |
| Tortilla | Nixtamalization + baking | Mexico | NR | n = 396<br>17% with AFs<br>3–385 μg/kg $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ | Castillo-Urueta, Carvajal, Méndez, Meza, and Gálvez (2011) |
| Tortilla | Nixtamalization + baking | Mexico | n = 9<br>100% with $FB_1$<br>0.2–1.8 μg/g | NR | Dombrink-Kurtzman and Dvorak (1999) |
| Tortilla | Nixtamalization + baking | Guatemala | n = 72<br>100 with $FB_1$<br>0.4–11.6 μg/g | NR | Meredith, Torres, De Tejada, Riley, and Merrill (1999) |

especially for malnourished children in Guatemala. Wall-Martínez et al. (2019) also reported the presence of AFs and FBs in tortilla samples in Veracruz, Mexico. Co-exposure to AFs and FBs and the exposure to FB analogues need much more attention in the field, as do their health impacts in Mexican and Central America populations (Gilbert Sandoval, Wesseling, & Rietjens, 2019; Torres et al., 2015).

More surveys in the field and of storage and end products are necessary in other countries of the region and should include other mycotoxins and the modified/matrix associated forms. It is also important to explore alternative analytical methods as proposed by Chavez, Cheng, and Stasiewicz (2020) that include near infra-red reflectance and fluorescence imaging. Other risk-assessment modeling approaches considering simultaneous mycotoxins could also provide further and more holistic information for taking measurements and devising specific mitigation actions (Battilani et al., 2020; N.; Liu et al., 2021).

## 6. Conclusions

Mexican and Central American populations are at a high risk of mycotoxin exposure and especially to AFs and FBs due to the large intake of products from crops that are prone to high contamination. Pre- and postharvest mitigation strategies as well as appropriate regulatory environments could help reduce exposure to the mycotoxins. The climate-change effect on crop safety is increasing, and therefore actions during pre-harvest are essential. At a postharvest level, several options have been explored. Some are more applicable at a lower scale while others still need further investigation and application in the region. However, the creation of awareness, training, and the communication of mycotoxin issues in Mexico and Central America are first steps towards the effectiveness of pre- and postharvest mitigation strategies. If farmers and vulnerable populations are aware of the health and nutrition implications of mycotoxin-contaminated grain and crops, they might enact or more regularly employ simple practices like kernel cleaning and avoid using contaminated kernels to feed livestock. At a country level, the creation of awareness could also aid in enforcing and monitoring regulations. Unfortunately, no, or very limited public information, including scientific studies or regulations were found for countries like El Salvador, Honduras and Nicaragua. Grain integrity and quality are basic traits that should be considered more in maize production, consumption and trade. Hermetic technologies can reduce fungal contamination and AF production during storage. Several methods to treat contaminated grain have been studied, but their application in the region is limited. Some of the main limitations are the potential effects on sensory traits of the food products and the cost and scalability of such technologies.

The nixtamalization process significantly reduces the amounts of AFs and FBs present in products like tortillas and fried snacks due to the physical removal of these toxins. Given the fact that nixtamalization contributes to the release of hidden FBs, monitoring of HFB is very relevant. Optimization of nixtamalization guidelines at both the household and industrial level need to be developed, since they may contribute to a decrease in or elimination of AFs and FBs in products.

## Declaration of competing interest

The authors reported no potential conflict of interest.

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